

## INFLUENCE OF CHEMICAL COMPOSITION OF ALGAL FOOD SOURCES ON GROWTH OF JUVENILE OYSTERS, *CRASSOSTREA VIRGINICA*

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### ABSTRACT

Two algal flagellates, *Dunaliella tertiolecta* Butcher and *Tetraselmis maculata* Butcher, harvested in the stationary phase from a semi-continuous carboy culture apparatus, were analyzed for dry weight, total carbohydrate, total protein, and total lipid. Each species was cultured in three different growth media. The growth response of *D. tertiolecta* was similar in all three formulations but populations of *T. maculata* were considerably limited in the reduced-nutrient medium, X<sub>1</sub>. Both algal species cultured in the X<sub>1</sub> medium had significantly greater dry weights and contained more carbohydrate and less protein than cells cultured in the standard formulation (E). A third formulation (N/P), in which all medium components were reduced except nitrate and phosphate, produced algae with reduced carbohydrate and increased protein as compared with E medium. The total lipid content of *D. tertiolecta* was significantly less than that of *T. maculata* regardless of the culture medium.

Algae cultured in the three formulations were fed to juvenile oysters, *Crassostrea virginica*. *T. maculata* was a consistently better food source than *D. tertiolecta*, indicating a probable causal relationship between algal lipid content and oyster growth. Growth of oysters fed algae cultured in X<sub>1</sub> medium was increased as compared with oysters fed algae cultured in E or N/P medium, suggesting a nutritional requirement for relatively more carbohydrate than protein as well. Results indicate that differences in growth media affect the gross chemical composition of algal food sources which alone can account for differences in algal nutritional value to *C. virginica*.

### INTRODUCTION

The laboratory or hatchery method of rearing molluscs in which brood stocks of adults are conditioned for spawning and induced to release gametes, and whose fertilized eggs are reared through larval stages to metamorphosis, requires that a suitable source of nutrition be available throughout the life cycle of the species that is being reared. This nutrition is derived most often from the culture of selected species of unicellular algae (see review, Walne, 1964; Ukeles, 1971, 1980; Epifanio, 1982), which are introduced into trays and tanks holding the molluscs. Other methods of obtaining algae have also been used (Glancy, 1965; Castagna, 1975), different sources of nutrition have been explored (Epifanio, 1979), and the development of artificial diets is in progress (Gabbott *et al.*, 1976; Langdon, 1983). From the earliest work in which it was recognized that unicellular algae are food sources for molluscs (Cole, 1936; Bruce *et al.*, 1939) investigators have queried why some species of algae are better food sources for molluscs than others. Certain factors related to the algal

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cell have been suggested as explanations, *e.g.*, cell size, cell wall composition, digestibility, the presence or absence of toxic metabolites, and chemical composition of the algal cell. None of these characteristics alone has offered an entirely satisfactory explanation. Although it seems that gross chemical composition (protein, carbohydrate, lipid) of different algae should be highly significant in affecting molluscan growth, research on this subject has yielded inconclusive results (see review, Webb and Chu, 1982).

The research reported here describes the manner in which the gross chemical composition of two algal species of close taxonomic standing can be induced to vary and how the chemistry of the algal food source influences the growth response of juvenile oysters, *Crassostrea virginica*.

## MATERIALS AND METHODS

### *Algal culture and population determination*

*Dunaliella tertiolecta* Butcher and *Tetraselmis maculata* Butcher, obtained from the Milford algal culture collection, were examined. These species, cultured axenically in an enriched sea water growth medium, E, have been in the collection for many years, and strains of these phytoplankters have also been maintained in a reduced-nutrient enriched sea water medium, X<sub>1</sub>, for several years. Strains cultured in a third formulation in which all medium components except nitrate and phosphate were reduced, N/P, were developed during the course of these experiments. Inocula for the growth curves reported in this study had been subcultured routinely in N/P for over one year. Medium formulations are shown in Table I.

Algae were cultured in the following types of Pyrex glassware: 20 × 150-mm screw-capped test tubes (with the liners removed) that were matched and calibrated for use as cuvettes in growth experiments; 125- and 500-ml Erlenmeyer flasks; and Fernbach flasks fitted with siphons and filling bell attachments to inoculate semi-continuous 18-liter carboy cultures (Ukeles, 1973).

TABLE I

*Formulations for algal growth media; final concentrations for 1 liter*

Component	Medium		
	X <sub>1</sub>	N/P	E
Sea Water*	500 ml	500 ml	500 ml
NaNO <sub>3</sub>	77.5 mg	300 mg	300 mg
KH <sub>2</sub> PO <sub>4</sub>	5.0 mg	20 mg	20 mg
NaFe Sequestrene	0.5 mg	0.5 mg	5 mg
THAM**	0.5 gm	0.5 gm	1.0 gm
Vitamin B <sub>12</sub>	0.8 µg	0.8 µg	3.0 µg
Thiamin HCl	0.08 mg	0.08 mg	0.3 mg
CuSO <sub>4</sub> · 5H <sub>2</sub> O	4.9 ng	4.9 ng	9.8 ng
ZnSO <sub>4</sub> · 7H <sub>2</sub> O	11 ng	11 ng	22 ng
CoCl <sub>2</sub> · 6H <sub>2</sub> O	6.5 ng	6.5 ng	13 ng
MnCl <sub>2</sub> · 4H <sub>2</sub> O	90 ng	90 ng	180 ng
NaMoO <sub>4</sub> · 2H <sub>2</sub> O	3.1 ng	3.1 ng	6.2 ng
Adjust pH to 8.0 and bring to volume of 1 liter with double glass-distilled water.			

\* Salinity 27–28‰.

\*\* Tris(hydroxymethyl)aminomethane.

Media were steam-sterilized in a Castle autoclave for 20 minutes (40 minutes for carboy media) at 15 psi pressure. Test tube and flask cultures were incubated in a GPI Model RI Incubator/Growth Chamber at 20°C, illuminated to about 300 ftC with cool-white fluorescent lights on a 12:12-h light/dark cycle. Carboys received constant illumination (300 ftC) at 20°C  $\pm$  1°C.

Culture densities for constructing growth curves were determined turbidimetrically with a Bausch & Lomb Spectronic 20 Spectrophotometer-colorimeter. Algal populations were also determined by microscopic counts in an Improved Neubauer Hemacytometer (Bright Line). Carboy cultures which had been harvested daily and replenished weekly with sterilized media for four weeks were selected for analyses. Samples of algae taken five days after the most recent addition of medium were subjected to dry weight determinations according to the method of Epifanio and Ewart (1977) and chemical analyses, as described in the next section.

### *Analyses of algal chemical constituents*

Analyses of total protein were conducted using a heated biuret-Folin (HBF) procedure modified from Dorsey *et al.* (1977, 1978), a method which provided increased sensitivity and a more stable end point as compared with the well-known method of Lowry *et al.* (1951). Cells were collected on a 25-mm glass fiber filter, and protein was extracted with appropriate reagents at 100°C for 100 minutes. Modifications from the published procedure primarily involved the concentrations and final volumes of reagents in the reaction tube (Table II). Sample reactions were read in a Beckman DB GT Grating Spectrophotometer. Protein nitrogen values were determined by interpolation from a standard curve obtained with prepared solutions of bovine serum albumin. Total protein was then calculated using a conversion factor of 6.25 generally accepted for most marine algal species (Dorsey *et al.*, 1978).

Carbohydrate determinations were made using a phenol-sulfuric acid method for analysis of algae reported by Kochert (1978) based upon procedures developed by Dubois *et al.* (1956). Algal culture samples were collected and washed with sterile isotonic NaCl by repeated cold centrifugation in an IEC Model Pr-2 centrifuge. Cells to be assayed were then homogenized in ethanol as in Myklestad and Haug (1972); this method was modified by using a Sonicor 50W ultrasonic bath. Prepared glucose solutions were assayed to construct a standard curve as recommended by Marshall and Orr (1962).

TABLE II

*Preparation of solutions for heated biuret-Folin protein assay*

Reagent	Concentration of stock solution	ml for intermediate working solution	ml for 100 ml final solution*	w/v concentration upon addition to reaction tube*
Na <sub>2</sub> CO <sub>3</sub>	20 g/100 ml	—	10 ml	2%
NaOH	40 g/1000 ml	—	10 ml	0.4%
NaKTartrate	20 g/100 ml	2 ml/10 ml combine**	1 ml	0.04%
CuSO <sub>4</sub> · 5H <sub>2</sub> O	5 g/100 ml	2 ml/10 ml		0.01%

\* This corresponds with "reagent f" of Dorsey *et al.* (1977).

\*\* To keep CuSO<sub>4</sub> · 5H<sub>2</sub>O from precipitating, it is necessary to dilute NaKTartrate to about 7 ml before adding Cu solution, mix thoroughly, and then bring to final volume of 10 ml with twice-distilled water.

Determinations of total lipid were conducted using the method of Mukerjee (1956) as adapted by Strickland and Parsons (1968). Cells for lipid analyses were collected, washed, and homogenized as in the carbohydrate procedure described above. A precipitate of algal material obtained by evaporating the ethanol from the homogenate was assayed, and extinctions were corrected with appropriate stearic acid standard and blank determinations.

### *Oyster feeding experiments*

Juvenile oysters, *Crassostrea virginica*, were grown in molluscan rearing chambers, described in Ukeles and Wikfors (1982), which provided a constant flow of filtered, UV-irradiated sea water with temperature adjusted to 26°C. Each day, sea water flow was interrupted for four hours during which oysters were permitted to feed upon algal food suspensions introduced into the chambers. Daily rations of algal food cultures harvested from carboys were equilibrated to the same cytoplasmic volume of 0.6 ml packed cells per chamber as determined by centrifugation in modified Hopkins tubes (Ukeles, 1973). Resulting daily nutritional inputs in terms of algal cell number, dry weight, protein, carbohydrate, and lipid were calculated for each algal food source. Differences between means of replicate chemical analyses and daily nutritional inputs were tested with a Z test ( $\alpha = 0.05$ ).

Each molluscan rearing chamber contained 50 juvenile oysters of similar initial size. Brood stocks of adult oysters obtained from a commercial landing at New Haven, Connecticut, were conditioned in the laboratory for spawning and induced to release gametes by warm-water stimulation (Loosanoff and Davis, 1963). Fertilized eggs were reared through setting in filtered sea water on a diet of cultured algae. Young juvenile oysters thus obtained were utilized in these experiments. Growth of oysters was determined weekly by weighing pooled groups of 50 live oysters from each chamber on a Sartorius top loading balance. Mean live weight per oyster was calculated for each population and plotted *versus* time in weeks.

## RESULTS

### *Algal growth populations*

Population growth of *D. tertiolecta* cultured in E, N/P, and  $X_1$  media was nearly identical (Fig. 1). The effects of various  $\text{NO}_3/\text{PO}_4$  ratios were investigated in a factorial design experiment where 16 combinations of  $\text{NaNO}_3$  (77.5, 150, 300, and 350 mg/l) and  $\text{KH}_2\text{PO}_4$  (5, 10, 20, and 25 mg/l) were included in the  $X_1$  basal medium. Population growth of *D. tertiolecta* was similar over this range of concentrations; representative growth curves in nitrate/phosphate ratios of 77.5/5, 300/5, 77.5/20, and 300/20 mg/l are shown in Figure 2. Increased concentrations of chelated iron (up to 1.5 mg/l) and vitamins ( $\text{B}_{12}$  up to 6.4  $\mu\text{g/l}$  and thiamine  $\cdot \text{HCl}$  up to 0.64 mg/l) were also tested in an  $X_1$  base formulation for possible growth-promoting effects upon *D. tertiolecta*. Again, no differences in the growth of *D. tertiolecta* were detected. Clearly, population growth of *D. tertiolecta* is not affected by the reduction of major medium components from E to  $X_1$  levels.

In contrast, *Tetraselmis maculata* demonstrated a considerable reduction of growth in the  $X_1$  formulation (Fig. 3). This difference did not appear as a diminished rate of logarithmic growth but, rather, as a decrease in the maximum population as compared with E medium. The addition of nitrate and phosphate to  $X_1$  medium in concentrations equivalent to those in the E medium (= N/P) produced a population density equivalent to that obtained in E medium (Fig. 3). Increasing the vitamin

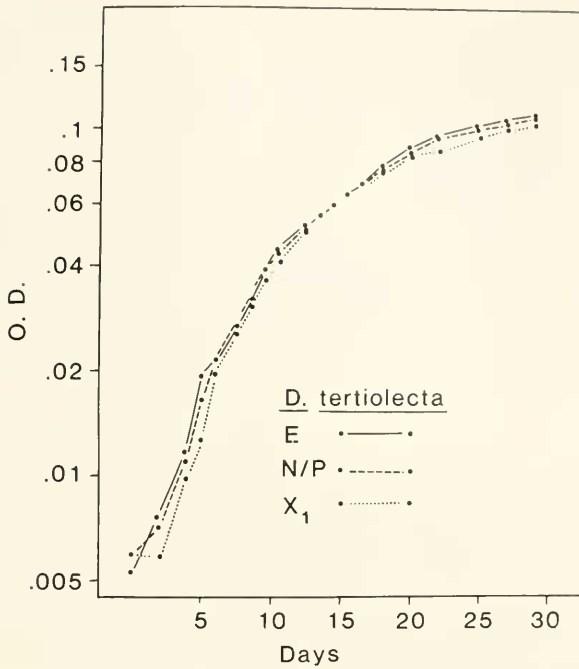


FIGURE 1. Growth curves of *Dunaliella tertiolecta* populations in three enriched sea water media: E, N/P, and  $X_1$ .

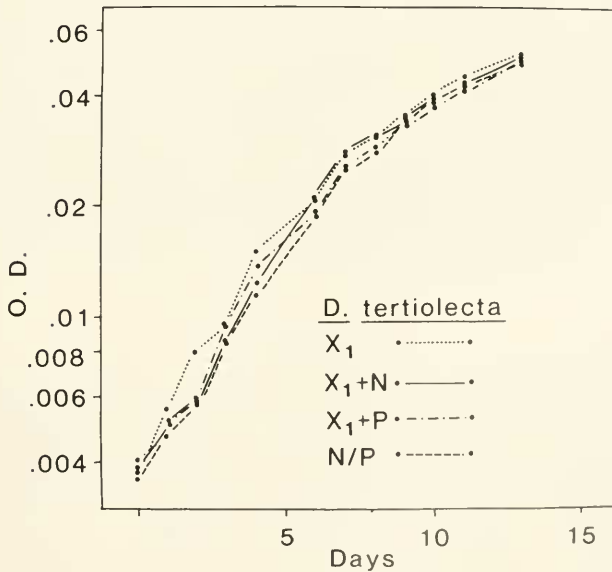


FIGURE 2. Growth curves of *Dunaliella tertiolecta* populations in  $X_1$  basal medium,  $X_1$  medium with increased  $\text{NaNO}_3$  (total  $200 \mu\text{g/l}$ ),  $X_1$  medium with increased  $\text{KH}_2\text{PO}_4$  (total  $20 \text{ mg/l}$ ), and N/P medium.



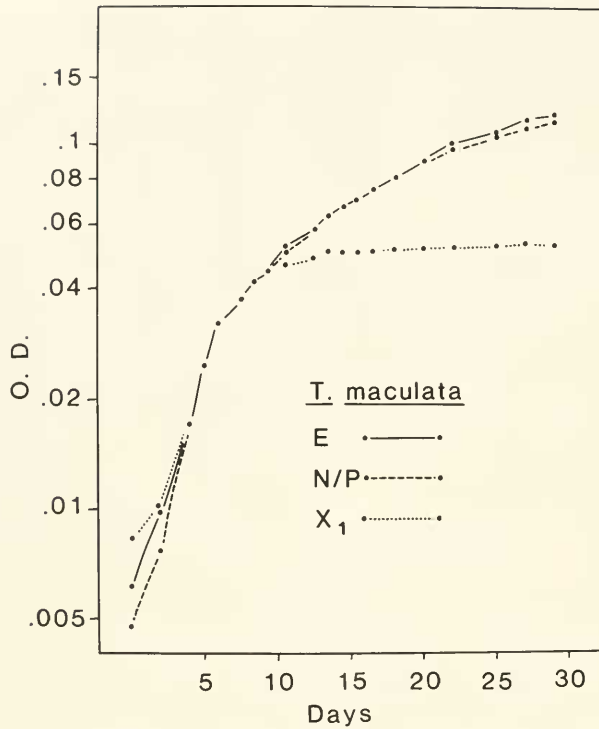


FIGURE 3. Growth curves of *Tetraselmis maculata* populations in three enriched sea water media: E, N/P, and X<sub>1</sub>.

concentrations in X<sub>1</sub> medium up to 6.4 µg/l for B<sub>12</sub> and 0.64 mg/l for thiamine · HCl did not affect any increase in *T. maculata* growth.

Dry weights of 10<sup>6</sup> cells of *D. tertiolecta* and *T. maculata* differed significantly ( $\alpha = 0.05$ ) when algae were cultured in the three formulations. *D. tertiolecta* cells cultured in X<sub>1</sub> medium had dry weights that were larger than those from E or N/P; the dry weights of cells from the latter two were not different statistically (Table III). Similarly, *T. maculata* cells had greater dry weights when cultured in X<sub>1</sub> medium than in E or N/P (Table III).

TABLE III

Gross chemical composition of algal cells

Species	Medium	Dry weight µg/10 <sup>6</sup> cells	% Total protein	% Total carbohydrate	% Total lipid
<i>Dunaliella tertiolecta</i>	X <sub>1</sub>	89.93	17.2	56.0	2.4
	E	78.26	39.4	31.1	2.0
	N/P	77.69	57.4	23.2	1.5
<i>Tetraselmis maculata</i>	X <sub>1</sub>	119.5	15.6	36.5	15.3
	E	110.9	31.0	23.9	8.3
	N/P	114.7	37.9	13.5	9.2

### Algal chemical composition

Although population growth of *D. tertiolecta* was not affected by the reduction of nutrients to concentrations contained in X<sub>1</sub> or N/P formulations, the chemical compositions of cells cultured in E, N/P, and X<sub>1</sub> media differed statistically. Differences in percent total carbohydrate between *D. tertiolecta* cells cultured in the three media were significant ( $\alpha = 0.05$ ) X<sub>1</sub> > E > N/P, and the same observation was made for *T. maculata* (Table III). Conversely percent total protein for each species cultured in the three media showed the opposite relationship ( $\alpha = 0.05$ ) N/P > E > X<sub>1</sub> (Table III). The percentage of the dry weight accounted for by the lipid fraction was significantly ( $\alpha = 0.05$ ) larger in *T. maculata* than in *D. tertiolecta* regardless of growth medium. Lipid content of both algae seems to be somewhat increased in X<sub>1</sub> over E and N/P media, but these differences could not be tested statistically.

### Oyster feeding experiments

Daily rations of algal food sources were adjusted to provide identical cytoplasmic volumes as previously described. Because algal cells cultured in different media were shown to have different dry weights and chemical compositions, it was necessary to compare daily rations for all algal food cultures in terms of number of cells, dry weight, and the chemical components in mg fed per day (Table IV). The outstanding difference in daily ration between *D. tertiolecta* and *T. maculata* involved the lipid component of the diet: oysters fed *T. maculata* received significantly more lipid than those fed *D. tertiolecta*. Daily rations of protein varied from 24.2 mg in X<sub>1</sub> to 68.2 mg in N/P for *D. tertiolecta* and from 23.9 mg in X<sub>1</sub> to 81.8 mg in N/P for *T. maculata*. Total carbohydrate in the algal diets ranged from 27.6 mg in N/P to 78.6 mg in X<sub>1</sub> for *D. tertiolecta* and from 33.4 mg in N/P to 55.9 mg in X<sub>1</sub> for *T. maculata*. Dry weights of 0.6 ml packed cells were higher for *T. maculata* than for *D. tertiolecta* with the *T. maculata* N/P diet having the largest dry weight, 248 mg, and *D. tertiolecta* in E medium having the smallest, 113 mg. Numbers of cells contained in the daily rations were very similar for the three *D. tertiolecta* diets ranging only from 1.44–1.56  $\times 10^9$  cells. The *T. maculata* diets showed more variation with a range of 1.28–2.16  $\times 10^9$  cells (in X<sub>1</sub> and N/P, respectively).

Growth responses of juvenile oysters to *D. tertiolecta* or *T. maculata* cultured in E, N/P, or X<sub>1</sub> medium are shown in Figure 4. Oysters exhibited increased growth when fed *T. maculata* as compared with *D. tertiolecta* regardless of the medium in which the cells were cultured ( $\alpha = 0.05$ ). After six weeks of observation, oysters fed either alga cultured in X<sub>1</sub> medium were larger than oysters fed algae cultured in E

TABLE IV

Composition of 0.6 ml packed cells for six algal diets fed to oyster populations daily

Algal species	Culture medium	Number of cells	Dry weight	Total carbohydrate	Total protein	Total lipid
<i>Dunaliella tertiolecta</i>	X <sub>1</sub>	1.56 $\times 10^9$	140 mg	78.6 mg	24.2 mg	3.34 mg
	E	1.44 $\times 10^9$	113 mg	35.1 mg	44.4 mg	2.22 mg
	N/P	1.53 $\times 10^9$	119 mg	27.6 mg	68.2 mg	1.74 mg
<i>Tetraselmis maculata</i>	X <sub>1</sub>	1.28 $\times 10^9$	153 mg	55.9 mg	23.9 mg	23.4 mg
	E	1.94 $\times 10^9$	215 mg	51.4 mg	66.8 mg	18.0 mg
	N/P	2.16 $\times 10^9$	248 mg	33.4 mg	81.8 mg	22.8 mg

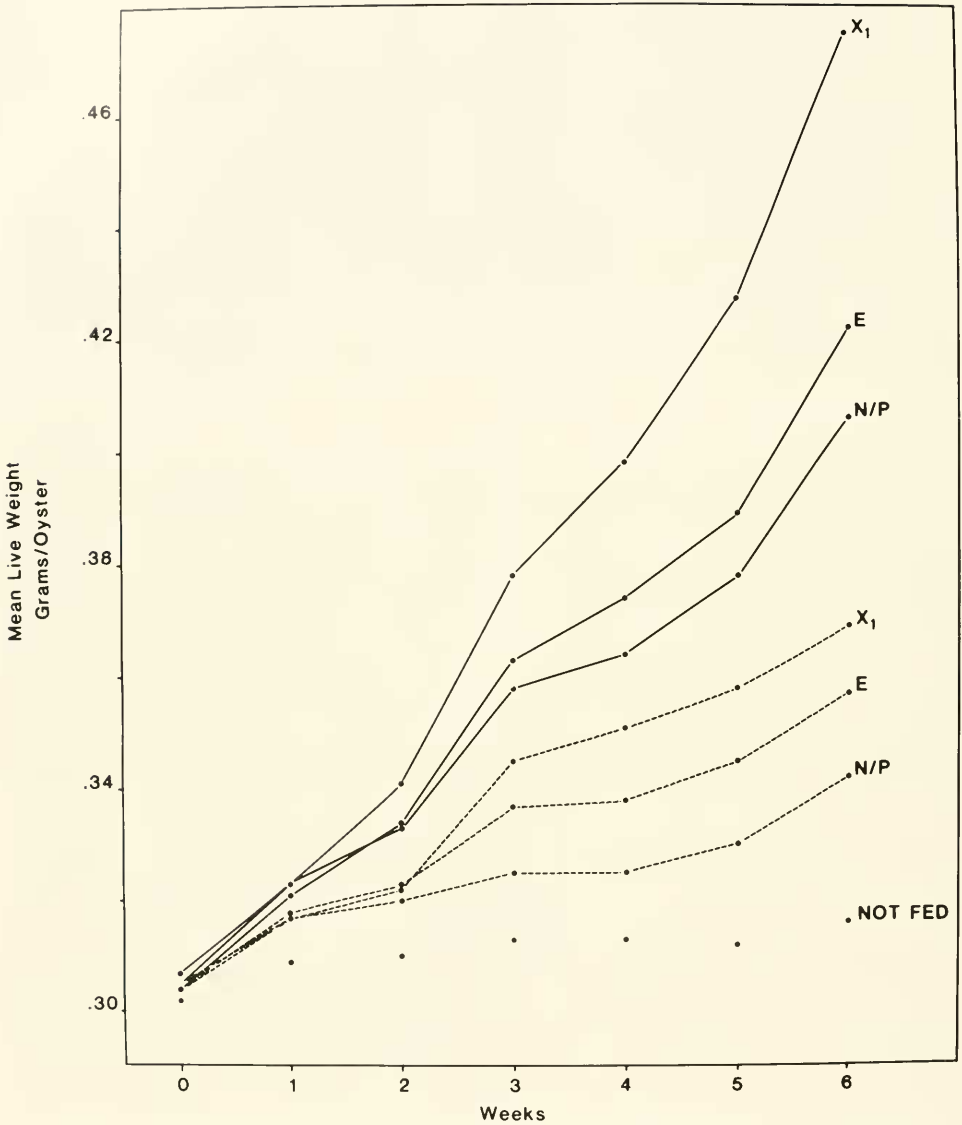


FIGURE 4. Growth of juvenile oysters, *Crassostrea virginica*, fed *Dunaliella tertiolecta* (dashed line), and *Tetraselmis maculata* (solid line), each cultured in three different medium formulations: E, X<sub>1</sub>, and N/P.

medium which, in turn, had grown more than oysters fed algae cultured in the N/P formulation. This relationship was consistent for both *T. maculata* and *D. tertiolecta*; however, statistical evaluation was difficult. Although data points shown in Figure 4 represent an average weight for 50 oysters from one chamber, these 50 were weighed as a pooled group; the small size and large numbers of the oysters rendered weighing individuals impractical. Over six weeks, calculated differences between mean weekly growth rates of oysters fed X<sub>1</sub>, E, or N/P cultures were not significant ( $\alpha = 0.05$ ) with the exception of *T. maculata* in which X<sub>1</sub> > N/P. This is not



surprising considering the somewhat hyperbolic shapes of the growth curves. Nevertheless, the consistency of the growth curves suggests that differences in growth of oysters fed either alga cultured in  $X_1$ , E, or N/P represent a genuine response to the different diets.

### DISCUSSION

In the present investigation, *T. maculata* and *D. tertiolecta* were compared as food sources for juvenile *C. virginica*. These algae are similar in that both are chlorophyte flagellates, ovoid or elliptical in shape with yellow-green chromatophores, and are of similar size (cell length of *D. tertiolecta* ranges from 6.4–11.7  $\mu\text{m}$  and *T. maculata* from 6.2–13.8  $\mu\text{m}$ ); the main difference is the presence of a medium rigid cell wall in *T. maculata* and a thin hyaline periplast in *D. tertiolecta* (Butcher, 1959). The algae were cultured in three different media formulations which resulted in differences in cell chemistry. These findings afforded a unique opportunity for evaluation of the significance of algal chemical composition in the nutrition of the filter-feeding bivalve, *Crassostrea virginica*. The highly controlled conditions provided by the use of axenic cultures and the chambers designed for rearing the bivalves served to eliminate many of the unknown variables that can affect data collected in feeding studies conducted under less rigorous experimental conditions.

*D. tertiolecta* tolerated a wide variation in nutrient enrichment of its growth medium; population growth showed little variation between the standard E formulation, the much reduced  $X_1$ , and the N/P formulation. In an earlier study it was also observed that two algal species used as molluscan food sources, *Monochrysis lutheri* Droop (= *Pavlova lutheri* comb. nov. Green) and *Phaeodactylum tricornutum* Bohlin can tolerate a substantial (50%) reduction of phosphate and nitrate concentrations in the growth medium (Ukeles, 1977). *T. maculata*, in contrast, responded to the low  $\text{NO}_3$  and  $\text{PO}_4$  concentrations of  $X_1$  medium with a reduced population; its needs for N and P were evidently higher than for *D. tertiolecta*.

Although the growth of *D. tertiolecta* populations was unaffected by variations in the three nutrient formulations of the growth medium, the chemical composition varied considerably. Stationary phase cells cultured in E medium and, more so, in  $X_1$  medium accumulated more carbohydrate than in the N/P medium. The composition of cells in N/P medium was higher in protein. The low carbohydrate and high protein contents found in N/P medium were similar to those reported for log phase *D. salina* cultured in an artificial sea water medium (Parsons *et al.*, 1961).

The protein concentration of both flagellates is lowest in the  $X_1$  medium and, in fact, the concentrations of protein in the three growth media are directly reversed to those of carbohydrates. Algae seem to have exhausted nitrogen for protein synthesis in the  $X_1$  medium and shunted fixed carbon to carbohydrate synthesis without suffering a reduction in final population as measured by optical density. This observation agrees with previous studies reporting that stationary phase algal cells, which accumulate carbohydrates (Handa, 1969; Myklestad and Haug, 1972; Chu *et al.*, 1982a; Loos and Meindl, 1982), are actually doing so in response to nitrogen depletion in the medium (Antia *et al.*, 1963; Werner, 1970; Hobson and Pariser, 1971). Stationary phase cultures of *T. maculata* from N/P medium, similar to *D. tertiolecta*, contained a high protein, low carbohydrate composition, agreeing with the results reported for analysis of log phase cultures of *T. maculata* by Parsons *et al.* (1961).

*D. tertiolecta*, as an oyster food, did not increase in nutritional value relative to *T. maculata* when cellular protein or carbohydrate was increased. Similarly, *T. maculata* did not diminish in value relative to *D. tertiolecta* when protein or carbohydrate

was decreased in the different growth media. Additional evidence that the amount of protein or carbohydrate fed per day does not limit the growth of juvenile *C. virginica* lies in the observation that the *D. tertiolecta* X<sub>1</sub> diet contained more carbohydrate than any *T. maculata* diet, and the *D. tertiolecta* N/P diet contained more protein than the *T. maculata* X<sub>1</sub> diet (Table IV). In no case was growth of oysters fed *D. tertiolecta* greater than that of oysters fed *T. maculata*. However, the lipid contents of *T. maculata* in all three diets were greater than those of *D. tertiolecta* by a factor of 10 (Table IV). Thus, there is strong evidence that total lipid, or a component of the lipid fraction, in *T. maculata* cells accounts for this alga's greater food value as compared with *D. tertiolecta*. Evidence that lipid content of a food source is critical in determining the growth response of oysters has been suggested in feeding studies with juvenile *C. virginica* offered different artificial diets (Castell and Trider, 1974), and the importance of lipids in the diet for larval bivalves has been well-documented (Millar and Scott, 1967; Helm *et al.*, 1973; Holland and Spencer, 1973; Holland, 1978; Chu and Dupuy, 1980; Chu *et al.*, 1982b). Components found in lipids extracted from algal cells showed that certain fatty acids, particularly those of the 6 w 3 group, are required by oysters (Langdon and Waldock, 1981; Langdon, 1982). The former study showed that a diet of cultured *D. tertiolecta* cells, fortified with encapsulated 22:6 w 3 fatty acid, produced better growth of *C. gigas* juveniles than did *D. tertiolecta* alone. It is possible, then, that *T. maculata* contains a required lipid component, perhaps a fatty acid, in large amounts as compared with *D. tertiolecta*. The greater nutritional value of *T. maculata* than *D. tertiolecta* is clearly correlated with the greater lipid concentrations in *T. maculata*.

It is noteworthy that both species cultured in the X<sub>1</sub> formulation in which the carbohydrate content was higher than the other two growth media offered the best oyster nutrition of the three media tested. Several investigators have implicated carbohydrates, particularly glucose, as being important in oyster nutrition (Gillespie *et al.*, 1964; Haven, 1965; Dunathan *et al.*, 1969). Flaak and Epifanio (1978), using *Thalassiosira pseudonana* as a food source, concluded that the growth of the oyster, *C. virginica*, was more rapid when algae were richer in carbohydrate than protein.

It has been known for some time that the chemical composition of freshwater algae can be affected by variation in environmental factors (Spoehr and Milner, 1948; Taub and Dollar, 1965; Saddler and Taub, 1972). Marine species, particularly diatoms, also have been shown to undergo changes in composition with alterations of environmental conditions. Observations of a phytoplankton bloom induced in a plastic sphere showed that with nitrogen depletion in the medium a significant change in the carbohydrate and protein composition of cells occurred (Antia *et al.*, 1963). A decrease in N/C ratio as a result of nitrogen deficiency has been observed in *Skeletonema costatum* (Holm-Hansen *et al.*, 1968), *Cyclotella nana*, and *Thalassiosira fluviatilis* (Hobson and Pariser, 1971). Myklestad and Haug (1972) found that with a depletion in nitrate there was a change in the protein/carbohydrate ratio in *Chaetoceros affinis*. Protein, carbohydrate, and lipid composition of two strains of *Phaeodactylum tricornutum* were found to vary in light and dark periods (Terry *et al.*, 1983) and it was also observed that temperature and light conditions will vary the composition of *Thalassiosira allenii* (Redalje and Laws, 1983). The present study has shown that the chemical composition of two closely related flagellates can depend upon the availability of nutrients in the growth medium.

The extreme variation in chemical composition of these algal species that was shown to be dependent upon nutrient availability poses a number of questions pertinent to ecological monitoring, as well as to the practical considerations of providing a source of nutrition for artificially reared filter-feeding species. High primary productivity

of marine and estuarine waters, as measured by algal cell numbers, chlorophyll content of sea water, dry weight of biomass, fixed carbon, or other methods currently employed by environmental biologists and oceanographers, may not necessarily reflect accurately the ability of these waters to support large populations in higher trophic levels. Predictions of harvests of commercially valuable species based upon "primary productivity" measurements alone could be overestimated if phytoplankton populations are deficient in some nutrient essential to the grazing species. Similarly, the nutritional values of cultured algae used to rear molluscan or other species in the laboratory or commercial hatchery could vary considerably depending upon algal species, medium formulations, growth phase of algae at harvest, or temperature and light conditions of culture.

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